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NUCLEIC ACID BASE SEQUENCE DETERMINING DEVICE

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NUCLEIC ACID BASE SEQUENCE DETERMINING DEVICE

[Kakusan enki hairetsu ketsutei sochi]

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[There are no amendments to this patent.]

Claims

1. A type of nucleic acid base sequence determining device characterized by the following facts: in this nucleic acid base sequence determining device, a detecting portion is set on the migration path of an electrophoresis device, and the nucleic acid fragments sequentially reaching the detecting portion are detected in real time, and the base sequence of the nucleic acid is determined; in this nucleic acid base sequence determining device, the electric field strength of the electrophoresis carrier in at least one site on the negative electrode side of the electrophoresis device from the detecting portion is lower than that of the detecting portion.

2. The nucleic acid base sequence determining device described in Claim 1, characterized by the fact that the cross-sectional area of the electrophoresis carrier perpendicular to the

migration path in at least one site on the negative electrode side of the electrophoresis device from the detecting portion is larger than that of the detecting portion, so that the electric field strength of the electrophoresis carrier is lower than the detecting portion.

3. The nucleic acid base sequence determining device described in Claim 2, characterized by the fact that an electrophoresis carrier is filled in a glass tube having a constricted portion to form the electrophoresis path, and the detecting portion is set at the constricted portion of the tube.

Detailed explanation of the invention

Industrial application field

The present invention pertains to a nucleic acid base sequence determining device. Especially, the present invention pertains to a nucleic acid base sequence determining device preferred for detecting nucleic acid fragments at a high resolution.

Prior art

In the prior art, determination of nucleic acid base sequence is performed as follows as described in "Saiho Kogaku", Vol. 1 (1982), pp. 79-87 and pp. 192-202: Maxam-Gilbert method or didexoy method is adopted to prepare nucleic acid fragments with various lengths and radio-isotope labeled. After the molecular weight is separated using the gel electrophoresis method, the migration pattern is visualized with auto-radiography for read. As shown in Figures 5(a), (b), as carrier of the gel electrophoresis, polyacrylamide gel in uniform-thickness plate form is used.

Recently, in the reference of J. Biochem. Biophys. Methods., Vol. 10 (1984), pp. 83-90, another scheme is described. In this scheme, instead of a gel with a uniform thickness, a tapered gel with thickness increased towards the positive electrode side, that is, at the end of the migration path, is used. The migration pattern is compressed near the end of the migration path, and the nucleic acid fragments with a wider molecular weight range are spread in the same gel. Figures 5(c), (d) illustrate the pattern.

Problems to be solved by the invention

The aforementioned two types of electrophoresis gels are dedicated to the method (autoradiography method) in which the migration pattern on the entire surface of the gel is read with autoradiography after end of electrophoresis. However, in this method, no consideration is made on the scheme in which a detecting portion is set on the migration path of the electrophoresis section, and the nucleic acid fragments sequentially arriving the detecting portion

with migration are detected, and the migration pattern is read in real time (real-time direct detecting method, see Figure 1). It thus has the following problems.

Usually, in the gel electrophoresis, when the nucleic acid fragments become longer, the spacing between the migration bands of the nucleic acid fragments with lengths different from each other by one base becomes narrower. On the other hand, when the nucleic acid fragments are detected, it is necessary to have the count number of the β-ray concentrated to higher than a certain level. Consequently, it is impossible to rapidly narrow the slit width and to reduce the steric angle that can effectively detect the β-line as the detection sensitivity falls. Consequently, as shown in Figures 5(a), (b), when a conventional gel with a uniform thickness is used, as the nucleic acid fragments become longer, two or more migration bands enter the slit at the same time, and it is impossible to separate the migration bands for detection. This is undesirable (see Figures 2(a), (b)). Also, as shown in Figures 5(a), (b), for the gel that tapers thicker at the terminal of the migration path, that is, on the positive electrode side, when the autoradiography method is adopted, although there is the advantage that more base sequences can be determined with a single gel, when the real time direct detection method is adopted, the spacing between the adjacent migration bands becomes narrower, so that it becomes difficult to separate and detect the long nucleic acid fragments. This is undesirable.

The purpose of the present invention is to provide a type of nucleic acid base sequence determining device using the real time direct detection method. For this type of device, the resolution of detection of the nucleic acid fragments can be increased without degrading the detection sensitivity, and it is possible to detect longer nucleic acid fragments, and it is possible to increase the number of base sequences that can be determined in a single round of electrophoresis operation.

Means to solve the problems

The aforementioned purpose is realized by stretching the electrophoresis pattern at the detecting portion so as to increase the spacing between two adjacent migration bands.

According to the present invention, the electrophoresis pattern is stretched by having the electric field strength of the electrophoresis carrier at the detecting portion higher than the portion on the negative electrode side from the detecting portion.

Also, the aforementioned higher electric field strength is realized by reducing the cross-sectional area of the electrophoresis carrier at right angle to the migration path at the detecting portion.

Operation

The electrical resistance of the electrophoresis gel is reciprocally proportional to the cross-sectional area of the gel. Also, because the same current flows at various parts of the same gel, the electric field strength in the gel is proportional to the electrical resistance, and the migration speed is proportional to the electric field strength. Consequently, the smaller the cross-sectional area of the gel, the larger the electric resistance, the higher the electric field strength, and the higher the migration speed. Here, by changing the cross-sectional area of the gel at the various portion of the same gel, it is possible to control the migration speed and to stretch/shrink the migration patterns of the various parts.

According to the present invention, the cross-sectional area of the gel perpendicular to the migration path at the detecting portion is smaller than that of the portion on the negative electrode side from the detecting portion. In this part, the electric field strength becomes higher, and the migration speed is higher, so that the migration pattern can be stretched. As a result, the spacing between the two adjacent migration bands can be spread.

As a result of the aforementioned constitution, even when the actual slit width is not reduced, the slit width still becomes relatively narrower with respect to the migration pattern, so that it is possible to increase the resolution without decrease in the detection sensitivity.

Application examples

In the following, an explanation will be given regarding an application example of the present invention with reference to the figures. Figures 1(a), (b) are a longitudinal cross-sectional view and front view of the device in this application example, respectively.

As shown in the figure, on one or both of two electrophoresis plates (1a), (1b) (one in the example shown in the figure), window (2), partition wall (3) and slit (4) are set for nucleic acid detection. Radiation ray detectors (5a)-(5d) are set for four types of nucleic acid bases, respectively. Electrophoresis gel (6) made of polyacrylamide gel as the electrophoresis carrier is filled in between two electrophoresis plates (1a), (1b). Its thickness is thinner in the detecting portion than in the detecting portion, on the negative electrode side and the cross-sectional area of the gel perpendicular to the migration path is smaller here. The electrophoresis gel can be prepared by processing two electrophoresis plates and a spacer for determining the spacing between them, and injecting acrylamide monomer in-between them, followed by polymerization. On the upper and lower ends of electrophoresis plates (1a), (1b), buffer containers (7a), (7b), negative electrode (8a) and positive electrode (8b) are set, respectively. Also, DC high voltage power source (9) is connected between the two electrodes to form a vertical type electrophoresis device. The outputs of radiation ray detectors (5a)-(5d) are input to signal processing device (10) for treatment, and are output with output device (11).

In the following, an explanation will be given regarding the operation of the present application example. First, a nucleic acid fragments mixture with various lengths radioisotope labeled with ^{32}P using Maxam-Gilbert method or didoxy method is injected to wells (12a)-(12d) set on the upper end of the gel for each of 4 types of nucleic acid bases. Then, a DC voltage (about 50 V/cm) from DC high-voltage power source (9) is applied between negative electrode (8a) and positive electrode (8b). As a result, the nucleic acid fragments migrate, so that the molecular weight is separated, forming migration band (13) for the nucleic acid fragments with the same length. The shorter the nucleic acid fragments, the higher the migration speed. Consequently, they reach radiation ray detectors (5a)-(5d) from the migration band of the shorter nucleic acid fragments. As a result, the migration bands arrive radiation ray detectors (5a)-(5d) from the migration band with the shorter nucleic acid fragments, and the β-ray emitted from ^{32}P is detected with the detector. The adjacent migration bands are separated for detection with slit (4). The signal output from the detector is sent to signal processor (10). Treatment is performed in consideration of which of the four types of the bases corresponds to the signal from the detector, and the electrophoresis pattern is read. In this way, in the device of the present application example, in real time of the electrophoresis, it is possible to determine the base sequence by reading the electrophoresis pattern directly in the real time during electrophoresis without using a photographic film.

However, as explained in the above, as the detected nucleic acid fragments become longer, the spacing between the adjacent migration bands, that is, the spacing between the migration bands of the nucleic acid fragments with length different from each other by one base becomes narrower. On the other hand, in order to detect the nucleic acid fragments, it is necessary to have the count number of the β-ray concentrated to above a prescribed level. Consequently, it is impossible to rapidly narrow the slit width and to reduce the steric angle for effectively detecting the β-ray. Consequently, in the conventional case when a gel with a uniform thickness is used, the length of the detected nucleic acid fragments is used, the length of the detected nucleic acid fragments becomes about 100-150 bases long. As a result, two or more migration bands enter the gel before the slit at the same time, and it is impossible to separate and detect the migration bands (see Figure 2(a)). In this application example, the gel thickness of the detecting portion is smaller than that of the portion on the negative electrode side from the detecting portion. More specifically, while the thickness of the well portion is 1.0 mm, the thickness of the detecting portion is 0.5 mm, and the cross-sectional area of the gel perpendicular to migration path (14) becomes smaller. As a result, the electric resistance of the gel in the detecting portion is higher. Consequently, when a constant current flows, the electric field strength rises, the migration speed becomes higher, and the migration pattern is stretched. Consequently, without actually reducing the slit width, the slit width can be made relatively

narrow with respect to the migration pattern. Consequently, it is possible to increase the resolution without degradation in the detection sensitivity (see Figure 2(c), (d)).

In addition to what is shown in Figure 1, the shapes of the gel also include those shown in Figures 3(a)-(c), and the same effects can be realized. Especially, compared with the detecting part, the shape of the gel on the positive electrode side of the migration path has no direct influence on the aforementioned effects.

In the following, an explanation will be given regarding another application example of the present invention. Figures 4(a), (b) are the longitudinal cross-sectional view and front view of the device in this application example, respectively. As shown in the figure, glass tube (15) has a constricted portion. The thickness of the glass in this portion is small enough to let the β-ray from ^{32}P well path through it.

It is used as the detecting window in this case. Said glass tube (15) is set for each of four types of nucleic acid bases. On the periphery of said portion as detecting window, annular slit (4) and radiation ray detectors (5a)-(5d) are set. In each glass tube (15), polyacrylamide gel is filled as electrophoresis gel (6). The diameter of glass tube (15) is finer (inner diameter of 2 mm) than the portion on the negative electrode from the detecting portion (inner diameter of 3 mm), so that the cross-sectional area of the gel perpendicular to migration path (14) as the detecting portion is smaller. On the upper end and lower end of glass tube (15), buffer containers (7a), (7b) as well as negative electrode (8a) and positive electrode (8b) are set, respectively, with DC high-voltage power source (9) connected to the two electrodes. The outputs of radiation ray detectors (5a)-(5d) are input to signal processor (10) and are processed. In addition, they are output with output device (11).

The operation of this application example is similar to that of said application example. In this application example, the mixture of nucleic acid fragments in various lengths is laminated on the upper end of the gel in each glass tube without a well. Also, in this application example, detection of the β-ray is performed from the circumference of the cylindrical gel. Consequently, compared with said application example in which detection is performed from one or both sides of a plate shaped gel, the steric angle of the β-ray detection is larger, and a β-ray count more then 3-fold or more than 2-fold is obtained. Consequently, in this application example, in addition to increase in resolution without degradation in detection sensitivity just as in said application example, it is also possible to increase the detection sensitivity. Also, by stretching the glass tube, it is easy to prepare the β-ray detecting window. This is another advantage.

Effect of the invention

According to the present invention, it is possible to stretch the migration pattern at the detecting portion. Consequently, even when the actual slit width is not narrowed, it is still

possible to make the slit width relatively narrower with respect to the migration pattern. Consequently, it is possible to increase the resolution without a decrease in the detection sensitivity.

Consequently, even when the spacing between the two adjacent migration bands becomes smaller in the nucleic acid fragments to be detected, the two migration bands can be separated and detected. Consequently, it is possible to increase the base sequence number that can be determined in a single round of electrophoresis.

Brief description of the figures

Figures 1(a) and (b) are longitudinal cross-sectional view and front view of the device in an application example of the present invention, respectively. Figures 2(a), (b) are longitudinal cross-sectional view illustrating the electrophoresis carrier of a conventional gel with uniform thickness and a diagram illustrating an example of output of the detector, respectively. Figures 2(c), (d) are longitudinal cross-sectional view illustrating the electrophoresis carrier in said application example and an example of the output of the detector, respectively. Figures 3(a), (b), (c) are longitudinal cross-sectional views illustrating modified examples of said application example. Figures 4(a), (b) are longitudinal cross-sectional view and front view of the device in another application example of the present invention. Figures 5(a), (b), (c), (d) are longitudinal cross-sectional view and migration pattern of the electrophoresis gel in the prior art.

Brief description of the reference numbers

1a, 1b	Electrophoresis plate
4	Slit
5a-5d	Radiation ray detector
6	Electrophoresis gel
7a, 7b	Buffer container
8a	Negative electrode
8b	Positive electrode
9	DC high-voltage power source
10	Signal processor
11	Output device
12a-12d	Well
13	Migration band
14	Migration path
15	Glass tube

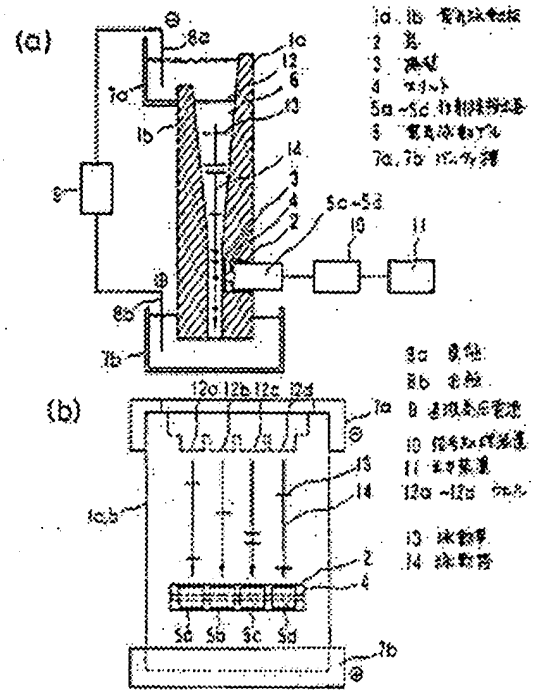

Figure 1

Legand:

1a, 1b	Electrophoresis plate
2	Window
3	Partition wall
4	Slit
5a-5d	Radiation ray detector
6	Electrophoresis gel
7a, 7b	Buffer container
8a	Negative electrode
8b	Positive electrode
9	DC high-voltage power source
10	Signal processor
11	Output device
12a-12d	Well
13	Migration band
14	Migration path

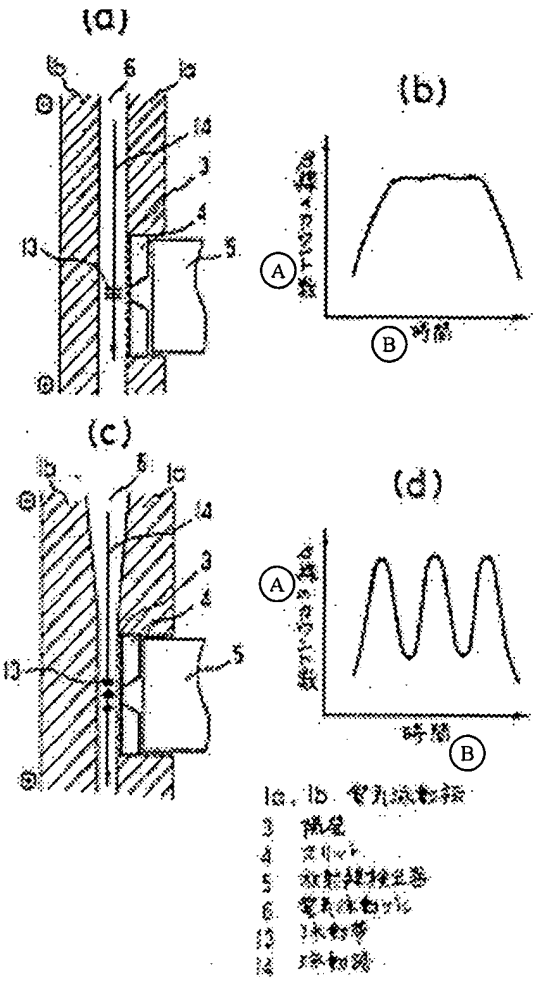

Figure 2

Legand:

A	1. β-ray count number
B	2. Time
1a, 1b	Electrophoresis plate
3	Partition wall
4	Slit
5	Radiation ray detector
6	Electrophoresis gel
13	Migration band
14	Migration path

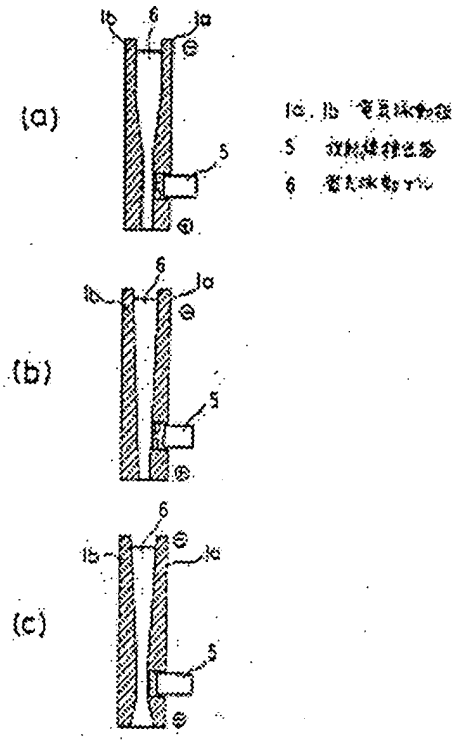

Figure 3

Legand:

1a, 1b	Electrophoresis plate
5	Radiation ray detector
6	Electrophoresis cell

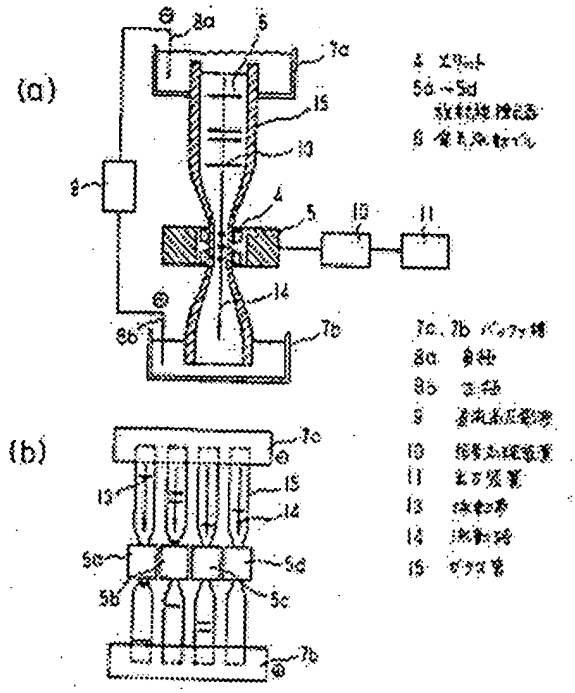

Figure 4

Legand:

4	Slit
5a-5d	Radiation ray detector
6	Electrophoresis gel
7a, 7b	Buffer container
8a	Negative electrode
8b	Positive electrode
9	DC high-voltage power source
10	Signal processor
11	Output device
13	Migration band
14	Migration path
15	Glass tube

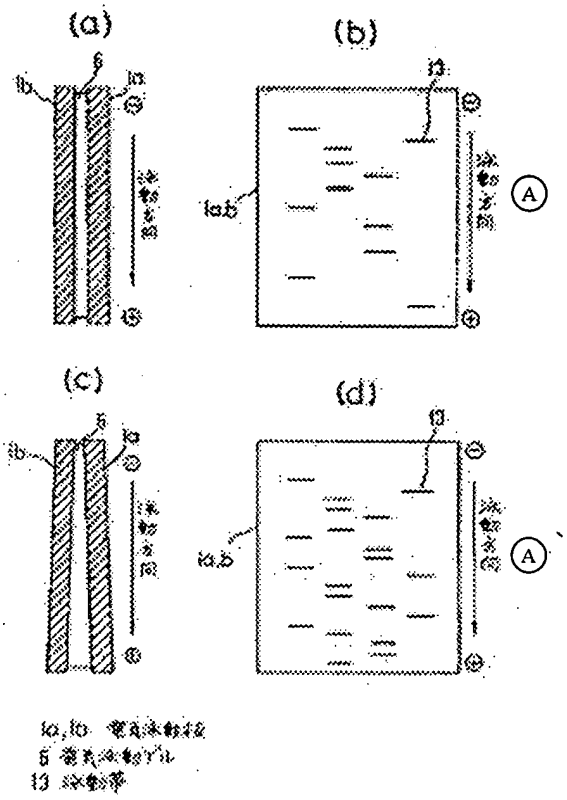

Figure 5

Legand:

A	Migration direction
1a, 1b	Electrophoresis plate
6	Electrophoresis gel
13	Migration band